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AN ECOLOGICAL STUDY OF DITYLENCHUS DIPSACI (KUHN)

FILIPJEV. IN A FIELD OF ALFALFA

by

Shu-ten Tseng

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

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Logan, Utah

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Shu-Ten Tseng

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INTRODUCTION

Ditylenchus dipsaci (Kuhn) Filipjev. is one of the most destructive nematodes to crop plants. It attacks and damages more than 300 species of cultivated and uncultivated plants. The nematode can be differentiated into many races, each of them having a preference for a group or even a single plant species.

Alfalfa stem nematode is one of the races in this species which attacks the above ground portion of alfalfa but does not infest the roots. Larvae of this nematode may migrate from the plant tissue to soil in moist conditions. These nematodes migrate actively to nearby plants or are carried by agricultural tools or wind to reach plants at some distance away, causing a new infection.

Godfrey (1923) reported the occurrence of this nematode in alfalfa and pointed out the potential danger of it in the Western part of the United States. It has been reported by other observers from widely separated areas of the U.S., especially in irrigated areas, and has become one of the major diseases in irrigated alfalfa production. Alfalfa stands of the infested field are thinned out gradually, rendering the crop unprofitable and the life of the crop is shortened by several years. McAllister (1959) obtained dry weight yields in varietal trials for a two year period as follows: Nemastan 14.8 tons; Lahontan 14.7 tons; Talent 12.0 tons; Ranger 11.9 tons; Grimm 9.4 tons; Narragansett 7.3 tons; and Nomad 6.1 tons. Nemastan and Lahontan were the varieties having resistance to the alfalfa stem nematode while the other varieties were susceptible.

The effective control of this disease seems dependent on the growing of resistant varieties and the prevention of the spread of this nematode.

A thorough understanding of the behavior of the alfalfa stem nematode may help in the development of an effective control measure. The present study was an effort to gain information about the biology of this nematode. The main purpose of this study was to determine the fluctuation in nematode population in soil where the infested alfalfa plants were growing, and its possible relationship to environmental conditions.

REVIEW OF LITERATURE

History of alfalfa stem nematode

The disease caused from the infection of D. dipsaci was first reported by Schwertz (1855). He recorded certain disease conditions of rye, oats, clover and other crops but did not observe the nematodes. The nematode was first described by Kuhn on Fuller's teasel, Dipsacus fullonum L. in 1857. He designated the name as Anguillula dipsaci Kuhn. He later discovered the same species on rye and a series of other cultivated plants which caused him to rename the nematode Anguillula devastatrix Kuhn. However, the change was not permitted by the rule of nomenclature. Since then, the nematode has been described by many observers in working with numerous kinds of plant species, and many synonyms have been designated for this nematode species.

Ritzema Bos (1888-1892), according to Thorne (1961), cleared the synonymy of D. dipsaci with the name of Tylenchus dipsaci by making transfers between certain of the thirty-eight known hosts. In this manner he demonstrated that the then so-called species Tylenchus hyacinthi, Tylenchus havensteini, and Tylenchus alli were actually Tylenchus dipsaci. Ritzema Bos was of the opinion that D. dipsaci included a group of races, each of which was responsible for the infection of a special group of host plants or even a single host species. In 1936, Filipjev designated Ditylenchus as the genus name.

In the United States, this pathogen was first reported by Bessey (1907), and its damage to alfalfa plants was first reported by Godfrey (1923) from several points in the western United States. Other observers

soon recorded it from widely separated areas of the country, especially in the irrigated areas of the Western States. Edwards (1932) reported its presence in New South Wales and Goodey (1933) stated that it was known to be in South Africa. In Argentina, Burkhart (1937) found it widely distributed and noted that Lorezetti had seen it as early as 1913.

Morphology

In the taxonomical position, the stem nematode, Ditylenchus dipsaci (Kuhn) Filipjev. belongs to Order Tylenchida, Family Tylenchidae, Genus Ditylenchus. It is slender, more or less filiform and pointed at both ends. The length of the nematode is 1.0-1.3 mm and its width approximately .03 mm. The head is distinctly offset, with three basal swellings. The median bulb is fusiform while the basal esophageal bulb is glandular with usually three prominent and two inconspicuous gland nuclei. The ovary is outstretched, sometimes reaching to the median esophageal bulb, but often is near the basal bulb. A rudimentary posterior uterine branch is present, extending about half way back to the anus. The vulva-anus distance equals $1 \frac{3}{4}$ to $2 \frac{1}{4}$ times the tail length. The terminus is acute, and the testis outstretched, with spermatocytes arranged in a single file except for a short region of multiplication. Bursa rise opposite the proximal ends of the spicula and extend about three-fourths the length of the tail.

Life cycle of Ditylenchus dipsaci

According to the observation of Yuksel (1960) on the life cycle of D. dipsaci, in onion tissue at 15 C, the total duration of the life

cycle ranged from 19 to 23 days with four moults and four larval stages. The egg stage lasted 7 days. There were apparent signs of cleavage of the egg cell only about 4 to 6 hours after an egg was deposited. And the development from the single cell stage to the full grown first stage larva took about 5 to 5 1/2 days. The first moult took place at this time inside the egg to become the second stage larva. The second stage lasted 2 to 2 1/2 days, the third stage 3 to 3 1/2 days, and the fourth stage 4 to 5 days before moulting. The hatching larvae are the second-stage larvae and are about 0.3 mm long. The various larval stages are distinguishable by their total length and by the nature and location of the genital primordia. In the third stage, the genital primordium becomes visible near the middle of the body and the sexes can be differentiated at the time. The fourth stage is known as the preadult, infective larva. It is at this stage in the life history that the nematodes have the remarkable ability to withstand adverse conditions of freezing and drying over a long period of time in dry alfalfa tissue.

D. dipsaci females begin to deposit eggs 3 to 7 days after the final moult. Females do not deposit eggs without mating, not even sterile eggs. Thus, mating is necessary for reproduction. A single male is able to fertilize more than one female. Egg-laying capacity of individual females ranges from 207 to 498 eggs. The females do not continue to lay eggs without feeding. Longevity of both the adult males and adult females in onion seedling was observed to be 45 to 73 days.

The influence of environment

Moisture. According to Wallace (1956) the movement of a nematode is wholly dependent on water. Unlike the earthworm which burrows through the soil, nematodes are confined to the soil pore spaces (Wallace, 1958). Consequently, the movement of a nematode in the soil and its invasion of a plant are very much influenced by the moisture in the soil as well as on the surface of the host plant. A thin water film on the plant surface is necessary for the nematode to move to the invasion site (Thorne, 1961), and infection and reproduction of D. dipsaci in alfalfa was observed to be favored by the increase of moisture (Barker, 1959; Barker and Sasser, 1959). Cairachi (1954) also observed that damage to tobacco by D. dipsaci was governed to a large extent by soil moisture.

In his experiment using various particle sizes of sand, Blake (1962b) found that in sand fractions with a particle diameter larger than 150 μ the migration speed of D. dipsaci was closely correlated with the number that invaded oat seedlings within 3 days after inoculation. Migration was fastest and invasion rates highest at the flex point of the moisture characteristic of each sand when the pores were draining. Seinhorst (1950) found that activity of D. dipsaci was inhibited at the moisture equivalent of soil. His data also showed that there was maximum activity in some soil types when the moisture content was about 120 to 135 percent of the moisture equivalent. The term moisture equivalent is used to express the moisture content after a saturated sample of the soil has been centrifuged at 1,000 g and is equivalent to a suction of about 500 cm of water. From the above facts Wallace (1963, p. 55) stated "that it is probable, therefore, that any

nematode activity in the soil which involved bodily movement will be at a maximum when the soil pores are empty but where water still remains at the points of contact of soil crumbs. Such conditions probably occur at moisture contents a little below field capacity."

Accompanying the variation of moisture, naturally, the osmotic pressure varies. The osmotic pressure may have some effect on the activity of the nematode. According to Blake (1961) movement of D. dipsaci larvae was not influenced by the osmotic potential of the sand solution until the concentration of the solution exceeded 0.3 M ($pF=3.87$). Then it decreased and movement ceased at 1 M ($pF=4.35$). Since in most fertile soil the salt concentration of the soil water does not exceed 0.05 M ($pF=3.05$), he concluded that nematode movement through a soil is normally governed not by the total potential but by the matric potential of the soil system. In his experiment, the larvae were killed in 1 M and 2 M urea solutions. However, an increase in the osmotic pressure with decreasing soil moisture had little effect on D. dipsaci because the moisture in most agricultural soils seldom reaches the potential of the wilting point of the plant ($pF=4.2$). Furthermore, the larvae of D. dipsaci will go into a state of quiescence when dried, and are highly resistant to desiccation.

Aeration. Ditylenchus dipsaci possesses the capacity to remain alive under anabiotic conditions to some extent (Filipjev and Schuurmans-Stekhoven, 1959). But Hastings and Newton (1934) found more quiescent individuals of D. dipsaci became active when they were placed in shallow dishes than in deep tubes with the same volume of water (c.f. Wallace, 1963). They showed that recovery decreased with increasing depth of water and that when the air over the water was

replaced by carbon dioxide there was no recovery of larvae from the state of quiescence. Twenty hours exposure to carbon dioxide appeared to kill the nematodes. Thus, aeration might be favorable to the activity and infection of this nematode.

Temperature. Ditylenchus dipsaci is very adaptable to various climatic conditions. Thorne (1961, p. 119) stated, "It is found from the cool, moist area of Northern Europe to the irrigated lands of the western United States, and from the hot desert soils of Southern California to the cold, high valleys of the Rocky Mountains."

Although the temperatures of 15 C and 7-13 C were found reported by Courtney and Latta (1943) and Cannis (1954) to inhibit the activity and infestation of D. dipsaci, respectively, the nematodes were not injured when infected alfalfa crowns were exposed throughout the winter in the mountains of Utah where night temperatures ranged from 5 to 20 C below zero for a period of four months (Thorne, 1961). Boshier and McKeen (1954) found that preadult larvae of D. dipsaci from narcissus bulbs withstood 20 minutes of -80 C treatment when dry. The nematodes were killed by this low temperature treatment when in a wet condition.

The optimum temperatures observed for D. dipsaci were 18 C for reproduction by Barker (1959); 10-20 C and 21 C for activity by Seinhorst (1960) and Sayre and Mountain (1962) respectively, and 15-20 C for mobility from Wallace (1961) and Blake (1962a). From these data it might be defined that the optimum temperature for D. dipsaci is around 15-20 C. However, studies on the temperature relations of nematode reproduction in plants are complicated because the plant itself is very much influenced by the variation of temperature.

Blake (1962b) showed that reproduction of D. dipsaci in oats was greater at 8 C than at 15 C. Furthermore, in carrot callus tissue cultures, Blake (1962b) found that the slow growth of the tissue at 8 C favored reproduction, whereas at 15 C the tissue grew quickly and inhibited nematode reproduction. Reproduction of D. dipsaci is therefore not only related to the temperature but also to the growth of the host. Wallace (1963) emphasized this by stating that infections of D. dipsaci are more severe in autumn sown than in spring sown oats in England; Autumn sown oats grow slower than the spring sown oats.

High temperatures that will inhibit the activity of D. dipsaci were found to be 38 C by Courtney and Latta (1934). As to the lethal high temperature Cairns (1953) showed that a narrow temperature range of 40-45 C caused death regardless of exposure time. A temperature of 43.5 C has been used for hot water treatment to control nematodes in narcissus bulbs (Green, 1964). In the treatment with high temperature Green found that acclimatization increased the heat resistance of the nematodes.

Soil type. The relationship of soil type and stem nematode is very complicated since the various chemical and physical properties of the soil arising from different soil types not only affect the activity of the stem nematode in soil directly but also the growth of the host plant which in turn will greatly influence the reproduction of the stem nematode.

The mobility of D. dipsaci was shown to be higher in sandy soil than in clay soil (Wallace, 1962). The mobility of the stem nematode in soil might influence the dispersion of the nematodes but is not

necessarily correlated to the injury of the host plants, since as reported in the work of Blake (1962a) the reproduction of D. dipsaci was greatly influenced by the growth rate of the host plants.

However, the infestation of D. dipsaci must have had some relationship with soil type. Seinhorst (1950) showed that infestations of D. dipsaci were found more frequent in clay soils in Holland. In a survey of the island of Goeree Overflakkee (Seinhorst, 1956) indicated that onion bloat was a persistent menace on all heavy clay soil, whereas, on light soils it only became important when onions were grown too frequently. He pointed out that the distribution of the nematodes in this island was not caused from the rotation practices of host and non-host crops. On the contrary, the rotation practiced in this island was formulated by the distribution and the degree of stem nematode infestation of onions.

Ditylenchus dipsaci population in soil
and its relation with damage

The density of a D. dipsaci population in the soil varies considerably with season. It usually shows a high density in the spring and autumn and a low density in winter and summer. Hutchinson et al. (1961) in their survey of plant parasitic nematodes in New Jersey noted that the populations ranged from very small in the winter months to very high in May and June. The mortality of D. dipsaci in fallowed land was high when the soil became frozen in the winter and the population decreased to an extreme low level (Lewis and Mai, 1960). However, the population density of the nematode fluctuated between rather narrow limits, in comparison to the other kinds of nematodes in soil, and the

limits of fluctuation of the D. dipsaci population density depended on soil type and availability of the host plants according to the studies of Seinhorst (1956, 1957). After the harvest of susceptible crops, the nematode population in sandy soil and light clay soil decreased more than in heavy clay soil. In heavy clays the degree of infestation showed a tendency to stabilize at a level of about 50 stem nematodes in 500 grams of soil. At a low degree of infestation (below 100 stem nematodes per 500 grams of soil) the population generally increased during summer whether host or non-host crops were grown. At the degree of infestation between 10 and 60 stem nematodes per 500 grams of soil the increase during the summer balanced the decrease during winter. On light soil, however, except when rye, oats or onions were grown, the degree of infestation mostly decreased during both summer and winter until there were only 5 nematodes per 500 grams of soil.

Very low population densities of D. dipsaci are capable of causing severe damage to the host plant. Seinhorst (1956) found that 10 stem nematodes in 500 grams of soil caused very serious damage to onions. At about 25 stem nematodes in 500 grams of soil there were no healthy plants left in the field. In alfalfa, Palo (1962) showed that 5 nematodes distributed at random on the soil within an area of 120 cm^2 did not show rapid multiplication. But the presence of 10 nematodes at random in an area of 120 cm^2 was enough to bring about an infestation which would be dangerous to a crop in a year.

The relationship between initial population density of D. dipsaci and the degree of damage to host plants has been discussed by several investigators. Sayre and Mountain (1962) found that the injury to

onion seedlings was linear for a range of 10 to 1,000 nematodes per pound of soil. Palo (1962) found a similar relationship for alfalfa. Seinhorst (1965) proposed the following mathematical model to describe the relationship between the density of stem nematode in soil and the proportion of exposed plants attacked: $Y = Z^p$ where Y = the proportion of the plants that were not attacked, p = the density of the nematodes and Z is a constant < 1 and equal to the proportion of the plants not attacked at a nematode density $p = 1$ (competition curve of Nicholson, c.f. Seinhorst, 1965).

Some aspect of behavior of *D. dipsaci*

Orientation and invasion. Bird (1959, 1960) found that many species of nematodes such as the larvae of Meloidogyne javanica and Heterodera schachtii larvae and adults of Pratylenchus minyus, preadult larvae of Pratylenchus sp were attracted by carbon dioxide. He concluded that carbon dioxide may play a part as a general, long range attraction to many nematodes under the conditions prevailing around plant roots. Klinger (1961) confirmed that the larvae of D. dipsaci were also attracted by carbon dioxide. These directed movements were also observed using a biological source of CO_2 formed by germinating seeds of white cabbage, onion, leek and red clover. The minimum concentration difference necessary for a directed movement to occur were in most cases between 0.08 percent and 0.15 percent per 1 cm. Thus, carbon dioxide given off by roots may be important in the locating of the host by the nematodes. However, other exudates may be necessary for the larvae to accumulate in a particular part of a host plant.

Invasion of D. dipsaci is not from the root but from the vicinity of the leaf base and growing point since the nematodes accumulate in this area (Hodson, 1926). A thin water film on the plant surface is necessary for the nematodes to move to the invasion site. The larvae of the stem nematode enter the alfalfa plant tissue directly from the epidermal cells of the shoot apex or leaf axil according to Krusberg (1961). Hanna and Hawn (1965) found that most of the alfalfa stem nematode penetrated the shoot apex and only a few were found in the cotyledons. The nematode in the cotyledons were thought to enter the stomatal openings when light was provided.

Actual feeding of D. dipsaci on the host plant cell has not been observed, but feeding on hyphae in the soil was observed by Linford (1937). Upon contact with the mycelium D. dipsaci pressed its head firmly against a cell and thrust its stylet vigorously and repeatedly until it either penetrated the cell or moved away. When penetration was accomplished, the stylet was thrust further forward into the cell and pulsation of the esophageal bulb began at once to suck up the cell sap.

Quiescence and longevity. On the life cycle of D. dipsaci, the preadult larvae have the remarkable ability to withstand adverse conditions of freezing and drying over a long period of time by lying quiescent in fragments of dried plant tissue. The longevity of this nematode varies greatly under different conditions. Low temperature and moisture conditions induce quiescence, thereby prolonging the life of the nematodes. The preadult larvae gather about the basal plates of dried narcissus bulbs to form cottony masses known as nematode "wool." Nematodes obtained from this "wool" were demonstrated to show

38 percent revival after 3 years storage at room temperature, 78 percent revival after 7 years storage at 2-4 C (Bosher, 1950) and withstood 20 minutes of -80 C treatment (Bosher and McKeen, 1954). Dormant preadult larvae were found alive in dry Dipsacus follonum after 23 years (Fielding, 1951). However, while active in plant tissue the longevity was as short as 45 to 73 days (Yuksel, 1961) under a constant temperature of 20 C. In soil in the absence of the host plant Goodey (1931) observed the nematode persisted only 1 1/2 years while Lewis and Mai (1960) showed 2 years of persistence in organic soil in southern New York.

Plant - Parasite relation

Symptom of the disease caused by D. dipsaci on alfalfa. The stem nematode usually attacks the succulent tissue of buds. A very characteristic symptom of the early stage of infestation is that the base of an infested young stem becomes enlarged, discolored and marked by fine transverse ridges or wrinkles (Thorne, 1961). Later on, infested portions with extremely shortened internode swell to form a characteristic gall. This enlarged node is occasionally produced on the plant several inches above the ground. In young seedlings the characteristic swelling occurs at the cotyledonary node. The growth of alfalfa is reduced and is associated with an abnormal increase of ramification and thickening of the stem base. Thus, in the field stunted patches of infested plants may be recognized. When the plants grow old the pith becomes affected and is destroyed so that the stems become hollow and easily broken off. The base of the matured stem which was infested by the nematodes is usually covered with brown lesions.

Histopathology. The histopathology of Ditylenchus dipsaci in alfalfa had been studied in detail by Krusberg (1961). In young seedlings, the nematodes had penetrated the terminal bud region 6 hours after inoculation. The nematode penetrated directly through the epidermis. Prominent enlargement, distortion and pulling apart of the cells were seen 12 hours after inoculation. Twenty-four hours after inoculation, vascular tissue was also affected; cells of the vascular bundle were separated from one another as were cortical cells in infected stems. Stem galling was easily visible without magnification. Eggs were seen in the gall cavities ten days after inoculation, and nematodes were seen in the cavities in the pith after 20 days. By this time often only five or ten nematodes were found in extensive galls. However, 45 days after inoculation epidermal cells around galls were greatly enlarged and gall cavities were full of nematodes and eggs. By this time the nematodes had also attacked the vascular tissues.

The nature of parasitism of stem nematodes is currently in dispute: Formerly the general nature of injury was thought to be the breakdown of the middle lamella by the pectinolytic enzyme of the nematode. However, Goodey (1948) suggested that plant hormones were involved in galls formed by plant parasitic nematodes. Krusberg (1963) also showed that pectinolytic enzymes were not of major importance in the disease caused by this nematode in alfalfa. The galling reaction of the tissue was most likely a plant growth regulation effect. Whatever the cause is, gall formation appears to be necessary for development of the nematodes (Rhode, 1965).

Resistance. Bovien (1955) defined the susceptibility of the plant as a condition when the nematodes are able to invade the tissues of the plant and establish themselves, and when the majority of them can reach sexual maturity or multiply and produce successive generation without a decrease in number, then, the plant is susceptible provided that such symptom which may be specific to the attack one developed as a result of the presence of parasites. Resistance is defined as a condition when the plant makes a normal growth in the presence of a pathogen and does not support the growth and reproduction of the parasite (Dropkin, 1955). It is also defined as tolerance when the plant not only has the ability to support the growth and reproduction of the pathogen but also succeeds in growing successfully itself in the presence of the parasite.

The larvae of D. dipsaci were observed to be able to invade resistant plants as well as susceptible plants. The tissue of susceptible plants invaded by the nematodes are usually swollen because of hypertrophy and showed apparent separation of cells, and the nematodes reproduce rapidly in the plant tissues. On the contrary, in resistant plants the tissue adjacent to the invaded cavities usually necrose because of hypersensitive reaction of the cells to the presence of the nematode secretions. There is less swelling and separation of cells, and the nematodes reproduce very little if any (Barker and Sasser, 1959). Thus, gall formation appears to be necessary for development of the nematode, and resistance is characterized by the failure of the nematode to complete its life cycle. The critical phase is the failure of egg production as shown by Grundbacher (1961). Larvae of D. dipsaci that invaded a resistant variety of alfalfa developed to adult stage, but produce none or only a very few eggs.

The characteristic of resistance of the alfalfa plant to D. dipsaci may be influenced by temperature. Grundbacher and Stanford (1962) found that resistance in Lahontan alfalfa was higher at 52 F than at 60 and 70 F. However, other resistant varieties such as Talent and selections from an introduction from Iran showed no temperature effect.

Inheritance of resistance to stem
nematode in alfalfa

Burkhart (1937) (c.f. Hare, 1965) was the first to undertake the breeding of alfalfa for resistance to the stem nematode. He found that resistance was dominant in the F_1 plants but could not be explained by a simple monofactorial system, nor did schemes with two or three dominant genes fit the data. Later, Ragonese and Marca (c.f. Grundbacher, 1962) reached similar conclusions and suggested a multifactorial explanation for inheritance of resistance. Generally, disomic inheritance was assumed for alfalfa. Stanford (1951) demonstrated the tetrasomic inheritance in alfalfa in his study of inheritance of flower color. Later in 1958 Stanford and Clement confirmed the tetrasomic inheritance from their cytogenetical studies of a haploid alfalfa plant derived from the California common variety. They concluded that common alfalfa is essentially an autotetraploid. In 1962, Grundbacher and Stanford reported that the characteristics of resistance to stem nematodes in Talent and a selection from an Iranian introduction were conditioned by a single dominant gene following the tetrasomic inheritance. Whether the characteristic of resistance in these two sources were on the same locus of the same chromosome was not known, though they suggested the possibility of a common origin by tracing the ancestors of these two varieties to the neighboring

countries of Iran and Iraq. They also reported a second pattern of inheritance of one or more minor factors in an introduction from Argentina and the variety Du Puits. The number of factors giving some selectable resistance appeared to be very small, and additive gene action was indicated.

Biological race

Ditylenchus dipsaci has a host range comprising over 300 plant species. There are evidences of the existence of biological races within this nematode species, each showing a different host preference. The problem of the existence of biological races in this species arose as early as 1888-1892 when Ritzema Bos cleared the synonymy of this species. Godfrey (1924) reported that the nematodes could be grouped according to host preferences into so-called biologic strains. Hodson (1926) also expressed the existence of biological races in D. dipsaci, but he suggested that these strains had varying power of adaptation to other host plants. Smith and Allen (1943) and Barker (1959) found that the teasel strain did not infect alfalfa and the alfalfa strain failed to reproduce in teasel. These nematodes from different sources could not be distinguished morphologically. Seinhorst (1957) was able to distinguish 11 biological races by inoculation on 9 plant species.

In a single host plant species more than one biological race may exist. Smith and Allen (1943) found that nematodes from a field in Reno, Nevada, were able to infect sweetclover, and white clover, while those from an alfalfa field near Minden, Nevada, were not capable of infecting sweetclover. Later, Smith (1951) showed that at least 2 biological races exist in alfalfa by inoculating with nematodes

collected from California, Nevada, Utah, and Virginia on a selection from the variety Nemastan. Also according to Allison (1956) the resistant variety Lahontan from Nevada showed only moderate resistance to the stem nematode in North Carolina. Goodey and Hooper (1962) found differences in the severity of attack on oats by populations of nematodes from different sites, but they indicated an alternate explanation that the inoculum from different sites varied in viability and may have been an indication of different biotypes.

Steiner was of the opinion that many of the forms which had hitherto been regarded as separate host strains of D. dipsaci are distinguishable by minute morphological characters and he regarded them as separate species (c.f. Bovein, 1955). Bovein (1955) pointed out this controversy could be solved by the cultivation of different races of D. dipsaci on an artificial medium with studies involving intercross between nematodes from different hosts.

Recently, Sturhan (1964) was able to successfully intercross between ten different combinations among 6 nematodes races from fodderbeet, alfalfa, red clover, "clover," rye, oats, and common valerian. The intercrosses were carried out using suitable host plants. Eriksson (1965) also demonstrated successful intercrossing between nematodes from alfalfa and those from red clover in callus tissue cultures derived from ley legumes species. There were abnormality in the shape of the body and tail in the hybrid populations. This suggested a remarkable genetic difference between the biological races. However, the successful intercross supports the theory that they belong to the same species (Sturhan, 1964; Eriksson, 1965).

Relation with bacterial wilt

In southern Alberta bacterial wilt Corynebacterium insidiosum McGull, and Ditylenchus dipsaci have been frequently found in the same stands of irrigated alfalfa (Hawn, 1963). The respective diseases they produce were often found in the same plants and symptoms were well developed and typical. In the U.S., bacterial wilt in alfalfa was also present in those areas where the stem nematode occurred (Smith, 1955). Hawn (1962) demonstrated that C. insidiosum was transmitted by D. dipsaci into alfalfa crown buds resulting in an increase of bacterial wilt in a wilt susceptible variety. He suggested that C. insidiosum was carried on the body of the nematodes rather than within it.

Spreading of the stem nematode

Dissemination of stem nematodes is mostly by passive transport. Palo (1962) found stem nematodes in alfalfa flower debris, i.e. in parts of pods, petals, etc., associated with samples of seeds but not in the seeds themselves. Thorne (1961) showed the following data on nematodes found in alfalfa seed lots.

Type of seed or screenings	Nematodes per pound
Recleaned seed ready for market	236
Thresher-run seed before cleaning	1,088
Fine seed passing through screens	1,134
Recleaned screenings	1,170
Fine screenings	16,629
Blowings from cleaning plant	4,398

These show that seed lots might be responsible for the dispersal of the nematodes from one place to another. Godfrey (1924) suggested that

the widespread occurrence of D. dipsaci on the Pacific Coast of the U.S. was due to wind blown infected seed of such plants as the false dandelion. Irrigation water is not only responsible for the spread of the nematodes to the vicinity of the infected plant but also to other remote fields through the irrigation canal as shown by the fact that 11,910 D. dipsaci individuals were found in 100 gallons of waste water from an infected field in Utah (Thorne, 1962). Transportation of hay and use of machinery may also cause spreading of the nematodes. Feeding of infected hay to livestock may introduce this nematode to other fields. Pieces of stems and buds often carry nematodes and are accidentally scattered in manure. Plowing and harrowing also spread nematodes in a field (Beaumont and Staniland, 1941; Seinhorst, 1950).

Control of stem nematodes in alfalfa

Though the nematodes had been found in the seed of field beans (Diercks and Klewitz, 1962) it had not been found in alfalfa seeds but in fruit materials in seed lots (Palo, 1962). The cleaning seed reduced the nematodes in seed lot to minimum numbers (Thorne, 1961). Thus, use of cleaned seed will at least largely slow down the spread of this nematode in alfalfa. Gostick (1963) had reported the effective control of seed borne alfalfa stem nematode with phorate (diethyl-s-(ethylthiomethyl) phosphorothiolothionate). Proper crop rotation will also successfully control the nematodes by growing non-host crops for two years or more. Sugar beets, grains, corn, potatoes, tomatoes, and other common crops are suitable non-host crops (Thorne, 1961). However, when alfalfa is planted again, the nematode usually reappears during the second or third year. The possibility of chemical control

of the stem nematode appears quite promising through the development of a systemic nematicide which will kill the nematode in the plant tissues. Barker and Sasser (1959) reported successful control of the stem nematode in alfalfa under controlled conditions with the experimental material o,o-diethyl-o-2-pyrazinylphosphorothioate (Zinophos) which is systemic in action. Zinophos is now registered as a nematicide (Good, 1965).

Cultivation of resistant varieties is the most important and the most effective measure in the control of the nematode. Extensive work on the breeding resistant varieties has been carried out. Some remarkable resistant varieties have been obtained.

1. Nemastan: About 1933-1936, an alfalfa was introduced from Turkistan, numbered F.C. 19304 that was found to be highly resistant to the stem nematode in Utah. It was increased and named Nemastan by G. Thorne. This variety also shows a high degree of resistance to bacterial wilt.

2. Talent: From an introduction from France (1935) numbered F.C. 19274, 1,500 plants which showed particularly good forage qualities and stem nematode resistance characteristics were selected for seed production and were released from Oregon Agricultural Experimental Station in 1952 as a nematode resistant variety. This variety is, however, susceptible to bacterial wilt.

3. Lahontan: This variety was developed by O. F. Smith in Nevada, by polycrossing progenies from Nemastan which showed a higher degree of resistance to both stem nematode and bacterial wilt with better seed and forage production than Nemastan. This variety is practically immune to the stem nematode and contains a higher degree

of resistance to bacterial wilt than the wilt resistant variety Ranger. It was released in 1955.

4. F. A. V. San Martin: This variety was developed in Argentina by Tome' from selections from the local varieties. It showed a higher degree of resistance to stem nematode and higher forage production than local varieties.

MATERIALS AND METHODS

An alfalfa field located near Smithfield, Utah, was selected for this study. It contained a six year old stand of Ranger alfalfa growing in Millville silt loam soil and was infested with the alfalfa stem nematode. The experimental design consisted of dividing the field into 16 sections and marking of a square plot 5 by 5 meters in each section. The distance between centers of any two adjacent plots was 12 meters.

Soil samples were obtained at two week intervals, from August 6, 1965, to June 25, 1966. Soil sampling depths were 0-10, 10-20, 20-30, and 30-40 centimeters. Three subsamples were taken from a plot at each sampling date using a soil auger of 10 cm in diameter. Subsamples were thoroughly mixed, and a 400 cc composite was collected in a plastic container. The remaining soil was immediately replaced into the hole. During the winter period a pick was used to break through the frozen soil to facilitate the use of the soil auger in sampling. Soil samples were processed to determine the nematode population.

Moisture content of the soil was determined from 4 of the 16 plots at the various sampling depths each sampling date. The percent moisture in the soil was determined by drying a 15 gram soil sample in the oven at 105-110 C for 48 hours. The moisture contents obtained were calculated to percent field capacity. The field moisture capacity of the soil was in turn determined by the following method: Soil was placed in plastic tubes (4 cm in diameter and 8 cm long) with a cloth

bottom. The tubes filled with soil were stood up in dishes containing water to a depth of 1 cm. After 24 hours they were placed on filter paper in a closed chamber and the excess water was allowed to drain out of the soil. The moisture content of the soil in these tubes was determined by the gravimetric method. The field moisture capacity of soil from the different depths determined by this method was:

Sampling depth (cm.)	Field moisture capacity (%)
0-10	30.1
10-20	28.5
20-30	27.1
30-40	29.0

Soil temperatures of each sampling depth were taken at approximately noon of every sampling date with metallic thermometers at the depths of 5, 15, 25, and 35 cm. A weather station set up in the field provided a continuous record of air temperature at 15 cm. above the ground and soil temperatures at the depths of 15 and 30 cm.

Soil samples were processed for nematodes within 24 hours. They were processed by the sifting and gravity, and the Baermann funnel methods (Thorne, 1961). Screen residues from 100 and 250 mesh sieves used in the sifting and gravity procedure, were collected for the Baermann funnel separation. Water was drained from the funnel after 24 hours to collect the nematodes. The nematodes collected were killed and fixed with FAA (Formaldehyde-acetic acid-alcohol) solution composed of the following parts: Water, 100; Ethyl alcohol, 20; 40 percent Formaldehyde, 16; and Glacial acetic acid 2 parts. Nematodes were placed on a watch glass having a subdivided bottom. The number of alfalfa stem nematodes were counted through a dissecting microscope.

RESULTS

The largest alfalfa stem nematode population was recovered from the 0-10 cm layer of soil (Figure 1). The number of stem nematodes decreased drastically as the sampling depth increased.

Two population density cycles were found (Figure 1). In the upper 10 cm layer of soil, the number of stem nematodes increased beginning the middle of August and approached a population peak in early September when the soil temperature was approximately 15 C. The number of nematodes decreased rapidly during the last part of September, when the soil temperature dropped to about 10 C. In the winter months, the population in the soil was consistently low, averaging less than 5 nematodes per 400 cc of soil. In the spring as the snow disappeared and the soil temperature rose to about 10 C, the number gradually increased. The nematode population reached a peak around the middle of May when the soil temperature approached 15 C. From early June the population dropped to a very low density again. The peak in May was substantially lower than that in August. The nematode density in the soil during the summer period would be expected to be very low as it was in the winter period.

The mode of change of the stem nematode density in the 10-20 cm depth was similar to that in the surface 0-10 cm layer of soil. However, the population in this depth approached its peak 2-3 weeks later than that of the upper 10 cm layer. The largest fall population in the 10-20 cm depth came at the beginning of October when the soil temperature at the 0-10 cm layer was about 10 C and the nematode

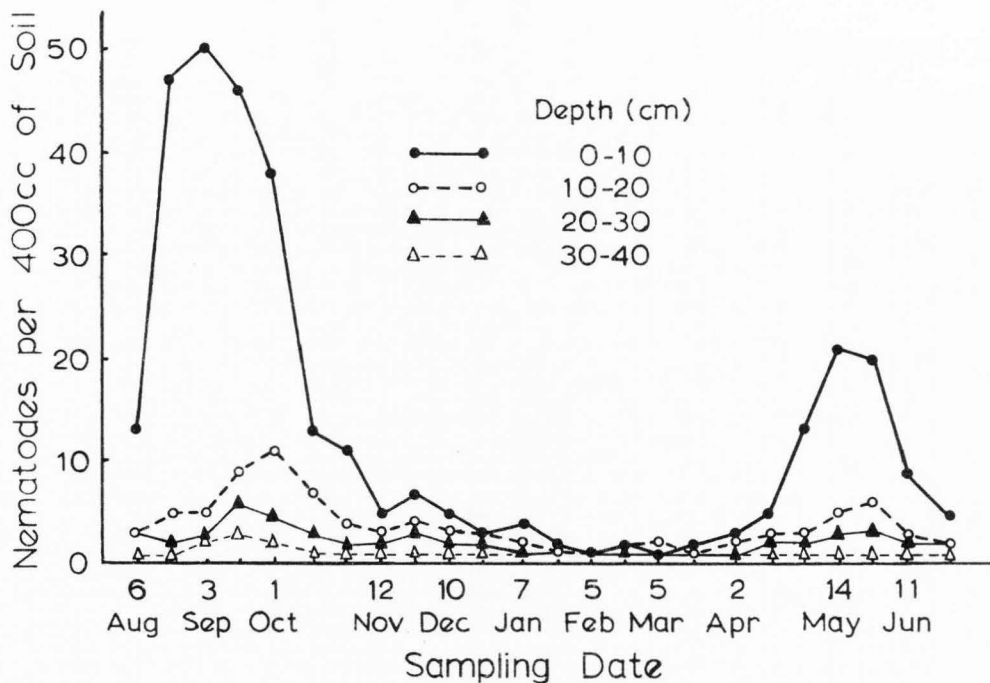


Figure 1. Population density of Ditylenchus dipsaci at different depths in the soil of an alfalfa field.

density at the 0-10 cm layer had already dropped. The spring peak appeared when the soil temperature of this layer was about 15 C and that of the 0-10 cm layer was above 15 C. The nematode population density in this soil layer was substantially lower in the spring than in autumn.

The stem nematode population density in the 20-30 cm and 30-40 cm depths also showed a tendency to follow a similar trend as for the depths of 0-10 and 10-20 cm but the nematode densities were much lower. At the 20-30 cm depth, a maximum of only 6 nematodes per sample was observed, while in the 30-40 cm depth the most nematodes obtained from any one sample was 3 nematodes per 400 cc of soil.

Soil moisture varied from less than 40 percent of field capacity in the fall to more than 100 percent of field capacity in frozen soil during the winter (Figure 2). The moisture content at the 0-10 cm soil layer showed the greatest variation while the range of variation decreased as the depth increased. The variation ranges for the soil moisture were: 0-10 cm depth, 35-108 percent; 10-20 cm depth, 46-94 percent; 20-30 cm depth, 53-91 percent and 30-40 cm depth, 53-87 percent field capacity. There were high and consistent soil moisture contents during the winter period, while they varied greatly among sampling dates during early fall and late spring. There was a noticeable decrease in soil moisture from August to October and from March to June. These were periods when the crop was actively growing.

Soil temperatures taken at each sampling depth for the various sampling periods, and the two-week period average temperatures from the continuous record taken at the depth of 15 cm and the air temperature at 15 cm above ground are shown in Figures 3, 4 and 5. The trends in temperature change were very similar. The temperature decreased

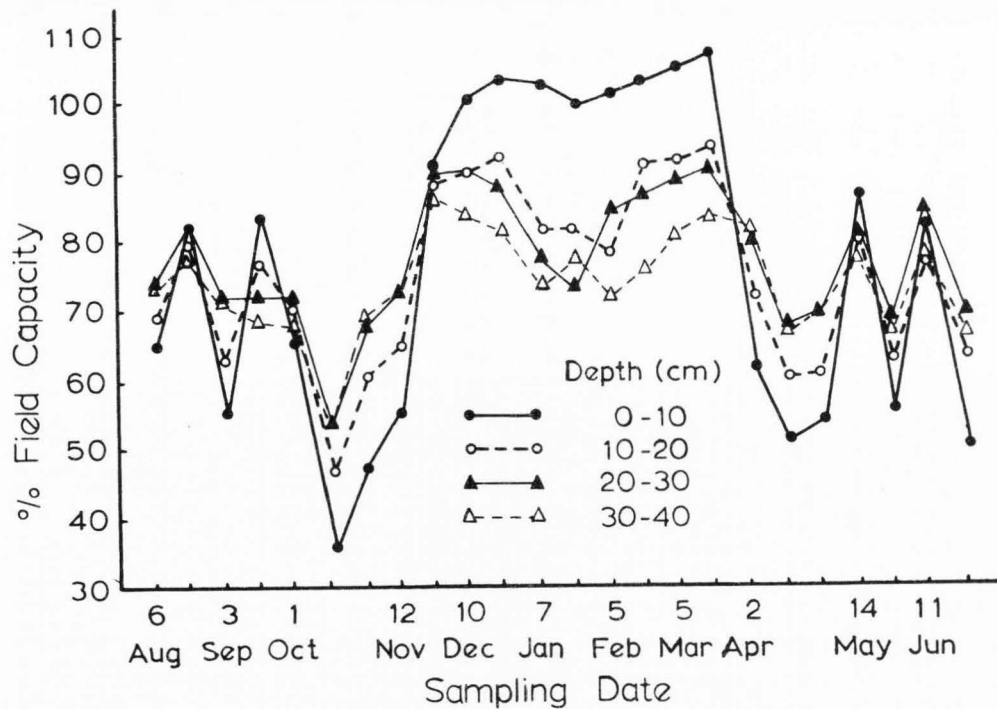


Figure 2. Soil moisture at different depths throughout the experimental period.

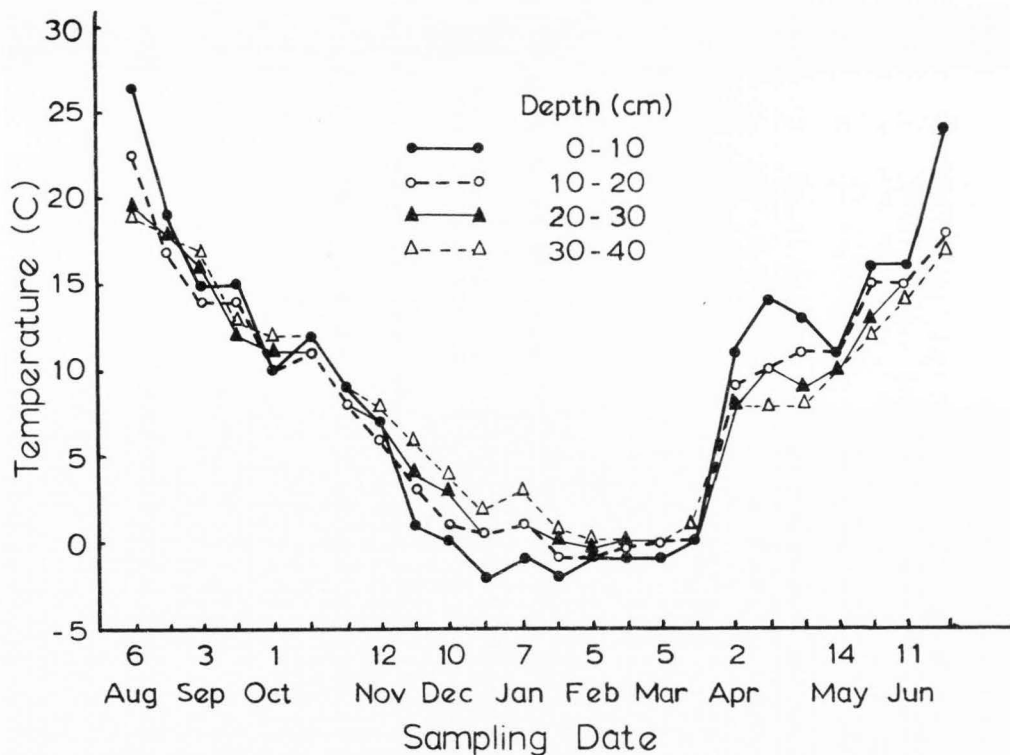


Figure 3. Soil temperature at different depths throughout the experimental period.

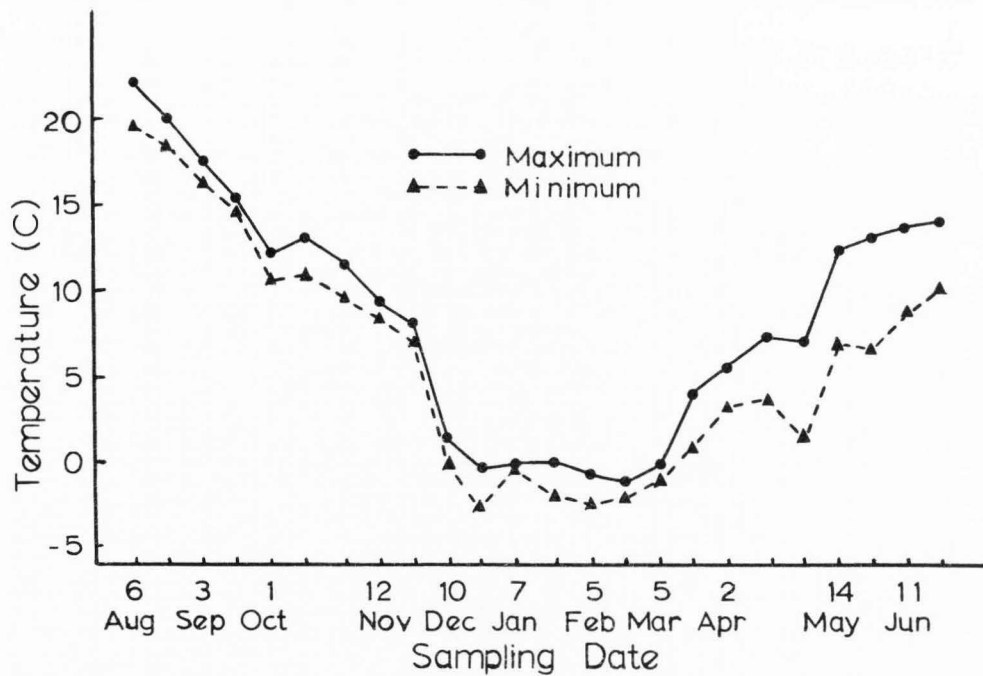


Figure 4. Average soil temperature at a depth of 15 cm over a two-week period prior to each sampling date.

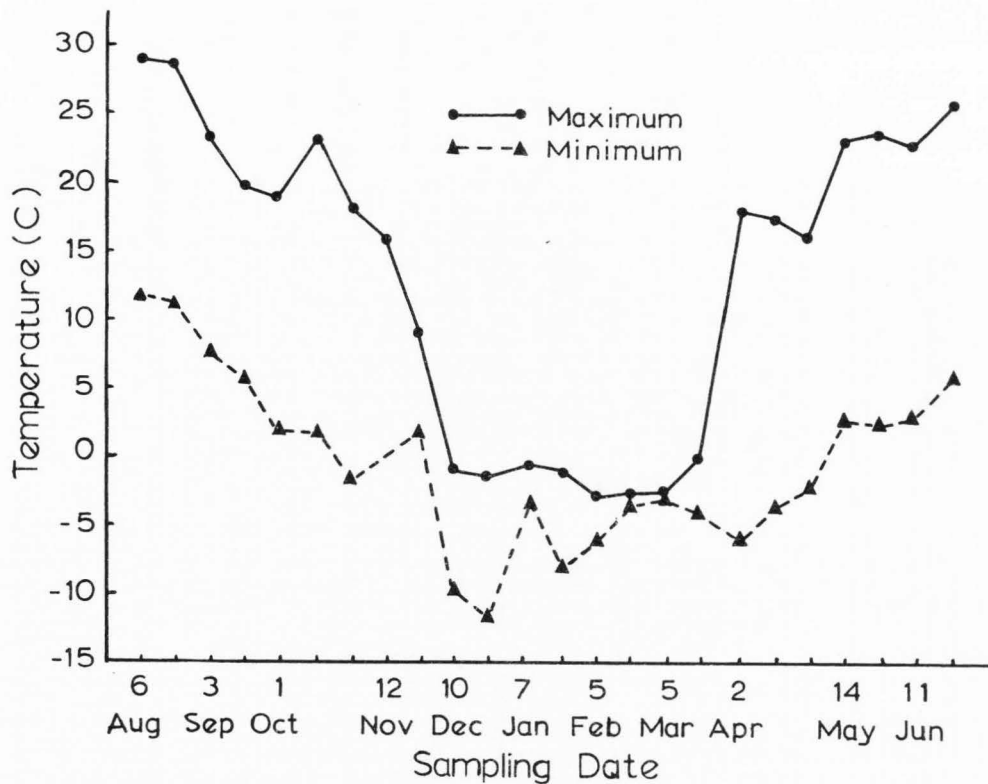


Figure 5. Average air temperature 15 cm above the ground over a two-week period prior to each sampling date.

from above 20 C at the beginning of August to below freezing beginning the middle of December. It remained low during the winter then rose rapidly beginning the end of March when the snow melted. Temperatures above 20 C and 15-20 C for the surface 10 cm layer and for the other lower layers of soil, respectively, were recorded in June.

Since there was a close relationship among the soil temperatures taken at the various sampling dates, average soil temperature from the continuous record and the air temperature at 15 cm above ground, only the soil temperatures taken at the different sampling dates at each sampling depth were used in the analysis of regression.

The following were taken as independent variables for an analysis of multiple regression with the number of nematodes found. (a) Soil moisture of each sampling depth determined at the sampling dates, (b) Season variable for which the first 16 samplings were set as 1 and the later 8 samplings as 2, (c) Deviation of the temperature from 15 C $[\text{Temp.} - 15]$, (d) Square of the above temperature deviation $[\text{Temp.} - 15]^2$, (e) Variable in time lag of 21 days, which the estimated stem nematode count 21 days before each sampling date, (f) Season X temperature deviation, and (g) Season X (temperature deviation)².

The regression analysis was conducted using a stepwise approach. All variables were used in the regression analysis. Then the variables were removed one at a time until the R^2 value started dropping.

Results of the analysis of regression are shown in Table 1. The predicted number of stem nematodes calculated from the models listed in Table 1 are shown in Table 2. The figure drawn from the predicted values for the 0-10 cm depth is similar to that from the observed data (Figure 6). The number of stem nematodes in the 0-10 cm layer of soil

Table 1. Result of the analysis of multiple regression between the nematode count and some environmental factors

Sampling depth	Source of variation	D.F.	Mean square	F
0-10 cm	Total	23	242.9493	
	Soil moisture (X_1)	1	631.1143	11.29**
	Season (X_2)	1	1747.1661	31.27**
	Temperature deviation (X_3)	1	1922.3260	34.40**
	Season x temperature dev. (X_4)	1	460.2981	8.24**
	Model	4	1131.5568	
	Error	19	55.8740	
$R^2 = 0.81001481$				
$Y_1 = 52.28 + 0.34 X_1 - 29.22 X_2 - 5.24 X_3 + 1.85 X_4$				
10-20 cm	Total	23	5.6069	
	Soil moisture (X_1)	1	6.9889	3.34
	Season (X_2)	1	10.2934	4.91*
	Temperature deviation (X_3)	1	40.6180	19.38**
	Time lag (X_4)	1	10.7239	5.12*
	Model	4	22.2856	
	Error	19	2.0956	
$R^2 = 0.69125069$				
$Y_2 = 2.38 + 0.06 X_1 - 1.76 X_2 - 0.34 X_3 + 0.44 X_4$				
20-30 cm	Total	23	1.5869	
	Season (X_1)	1	4.4647	5.14*
	Temperature deviation (X_2)	1	5.1363	5.92*
	Season x temperature dev. (X_3)	1	0.9045	1.04
	Model	3	6.3795	
	Error	20	0.8681	
$R^2 = 0.52434397$				
$Y_3 = 5.80 - 1.62 X_1 - 0.29 X_2 + 0.09 X_3$				
30-40 cm	Total	23	0.2591	
	Season (X_1)	1	0.2139	1.68
	Temperature deviation (X_2)	1	0.5433	4.26*
	(temperature deviation) 2 (X_2^2)	1	0.3459	2.71
	Time lag (X_3)	1	0.9211	7.22*
	Model	4	0.8837	
	Error	19	0.1276	
$R^2 = 0.59323299$				
$Y_4 = 1.54 - 0.23 X_1 - 0.16 X_2 + 0.01 X_2^2 + 0.47 X_3$				

*Significant at 5 percent level.

**Significant at 1 percent level.

Table 2. Observed and predicted number of the stem nematodes in various depths of soil (nematodes/400 cc of soil)

Sampling date	0-10 cm		10-20 cm		20-30 cm		30-40 cm	
	Observ.	Pred.	Observ.	Pred.	Observ.	Pred.	Observ.	Pred.
Aug. 6	13	5.7	3	3.4	3	3.3	1	1.1
Aug. 20	47	37.2	5	5.9	2	3.6	1	1.2
Sep. 3	50	41.4	5	6.0	3	4.0	2	1.4
Sep. 17	46	51.3	9	7.4	6	3.6	3	2.0
Oct. 1	38	28.0	10	6.5	5	3.4	2	2.4
Oct. 15	13	24.3	7	5.6	3	3.4	1	1.8
Oct. 29	11	18.8	4	3.9	2	2.8	1	1.5
Nov. 12	5	14.7	3	3.6	2	2.5	1	0.9
Nov. 26	7	6.3	4	3.4	3	2.0	1	0.9
Dec. 10	5	6.0	3	3.3	2	1.8	1	0.9
Dec. 24	3	1.4	3	3.3	2	1.3	1	0.9
Jan. 7	4	3.4	2	2.8	1	1.4	1	0.9
Jan. 21	2	-1.0	1	1.7	1	1.2	1	1.0
Feb. 5	1	3.1	1	1.0	1	1.1	1	1.0
Feb. 19	2	3.6	2	1.3	1	1.2	1	1.0
Mar. 5	1	4.2	2	1.8	1	1.2	1	1.0
Mar. 19	2	6.7	1	0.2	1	1.0	1	1.0
Apr. 2	3	7.5	2	1.9	1	1.8	1	0.9
Apr. 16	5	9.5	3	2.0	2	2.0	1	0.9
Apr. 30	13	9.1	3	2.7	2	1.9	1	0.9
May 14	21	16.9	5	3.5	3	2.0	1	1.1
May 28	20	10.6	6	4.5	3	2.4	1	1.2
Jun. 11	9	20.0	3	6.0	2	2.6	1	1.5
Jun. 25	5	-3.3	2	3.6	2	2.2	1	1.4

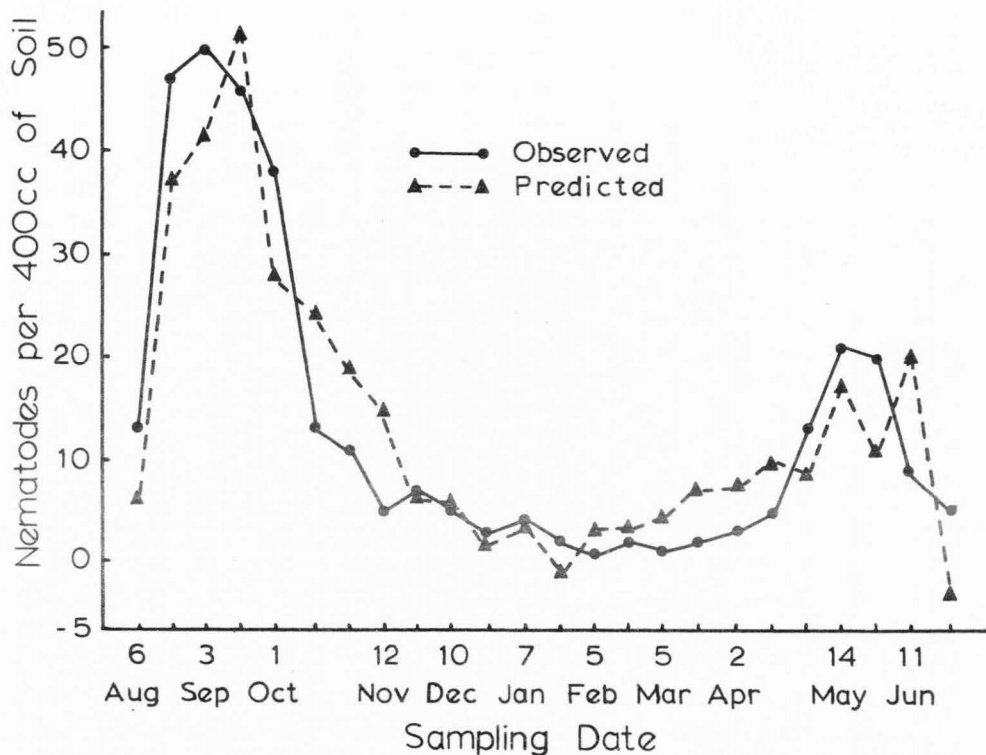


Figure 6. A comparison of the observed and predicted population density of Ditylenchus dipsaci in the 0-10 cm depth throughout the experimental period.

were mostly affected by the soil moisture, change of season, temperature deviation, and the interaction of season and temperature deviation. The number of nematodes increased with the increase in soil moisture. From the model, for each 1 percent increase in moisture capacity there was an increase of about 0.34 nematodes per 400 cc of soil. The number of nematodes found during the spring was less than found in the fall by approximately 29 nematodes per 400 cc of soil. Population density also decreased with the increase of the deviation of soil temperature from 15 C with a 5 nematode decrease for each 1 C increase in soil temperature above 15 C. There was a significant influence of the interaction between season and temperature deviation on the nematode count due to the different manner in which the temperature changed during the two seasons. In autumn, the soil temperature decreased while it increased during the spring.

For the nematode density at 10-20 cm, soil moisture was less important (the F value for the mean square due to moisture is very close to significant level though not significant), than at the 0-10 cm depth, although it still showed a substantial influence. Similar to the situation of 0-10 cm, season and temperature deviation showed a significant influence to the stem nematode density in this depth. There were approximately two more nematodes, per 400 cc of soil, found during the spring than in the fall. The nematode number decreased with the increase in temperature deviation from 15 C. The interaction of season and temperature deviation was not important for the stem nematode density in this depth. However, the time lag variable of 21 days showed a substantial influence on the number of nematodes in this depth of soil. It implies that at this depth the nematode density at sampling

time was correlated with the environmental conditions three weeks before, when the stem nematode density at 0-10 cm depth was in the population peak period. After the population peak period, the soil temperature was decreasing to below 15 C in the fall while it was increasing rapidly above 15 C in the spring. The maximum and minimum temperatures during this period were likely to depart more from the suitable temperature at 0-10 cm than at the deeper depths. It was possible that these conditions caused a migration of nematodes from the surface layer to a deeper depth of soil, or there was an increase in the hatching of eggs in the soil.

Soil moisture was not important in influencing the nematode density at depths of 20-30 and 30-40 cm. Temperature deviations were very important in the determination of nematode density at depths of 20-30 and 30-40 cm. The variable of season did not show a significant influence for the 30-40 cm depth, but was significant for the 20-30 cm depth. However, though significant, the average difference between seasons for the 10-20 cm and 20-30 cm depths was less than 2 nematodes per 400 cc of soil. The variable of time lag was significant for the 30-40 cm depth while it was insignificant in the 20-30 cm depth. At the depth of 30-40 cm, there was virtually no difference in the number of nematodes between seasons though there was a detectable relationship with the environmental factors as shown in the regression analysis (Table 1).

DISCUSSION

The population of the alfalfa stem nematode in the soil on which alfalfa plants were growing fluctuated greatly with the change of seasons. However, the range of variation was rather narrow and is in agreement with the results obtained by Seinhorst (1956). For the depth of 0-10 cm, where most of the stem nematodes were found, the range was from 50 nematodes per 400 cc of soil in the autumn to 1 nematode per 400 cc of soil in the winter. The population density of the stem nematode reached a peak between late August and early September and another peak the middle of May. These results agree with other observations such as those of Hutchinson et al. (1961) in which they observed a high population of Ditylenchus dipsaci in the soil in May and June in New Jersey. The population peak in autumn was substantially higher than in spring.

Cariachi (1954) observed that the damage of D. dipsaci to tobacco plants increased with an increase in soil moisture. Wallace (1962) also observed the increase of D. dipsaci in the soil of the infected oat plot with the increase of rainfall and a decrease with the following drying up of the soil. In the present study the stem nematode showed a positive correlation with soil moisture content. The moisture content in soil may not be the direct cause of the increase of the stem nematode in soil but rather an indicator of such a change. There was a possibility that the stem nematode in the soil migrated from the alfalfa plant tissue in which they reproduced and moisture was needed for the migration. If this was true, rainfall or irrigation water would provide

the needed moisture, also, either one would increase the moisture content of the soil. Another possibility for the increase in the stem nematode population was that the improved soil moisture favored the hatching of the nematodes from eggs that were present in the soil and plant debris. However, no evidence of recovery of eggs from the soil was obtained. On the contrary, Wallace (1962) and Webster (1964) demonstrated that D. dipsaci migrated from the onion and narcissus plant tissues, respectively, under moistened conditions.

The population density in the 10-20 cm layer approached its peak 2-3 weeks later than in the 0-10 cm layer when the temperature was well below 15 C in autumn and above 15 C in spring. This significant time lag might indicate that the nematode in the lower layer of soil probably migrated from the upper layer of soil. Wallace (1961, 1962) observed the directed movement of the nematodes caused from the temperature and moisture gradient in soil. Soil moisture influenced the migration of the nematodes from the upper to the lower soil layer. Soil moisture showed a substantial correlation with the nematode density in the 10-20 cm layer of soil. At depths of 20-30 and 30-40 cm, soil moisture was not an important factor influencing the nematode population density. The moisture variation range in the 20-30 and 30-40 cm depths were substantially narrower than in the 0-10 and 10-20 cm layers. The nematodes did not migrate very deep into the soil as shown in Figure 1. Most of the nematodes found were in the depth of 0-10 cm, there being less than 6 nematodes per 400 cc of soil when the depth was more than 20 cm while 50 nematodes per 400 cc of soil were found at 0-10 cm depth.

Temperature might be the most critical factor on the fluctuation of the stem nematode density in the soil. Both in autumn and in the spring, the nematode density reached its peak when the soil temperature was around 15 C. The larger the deviation from this temperature, the fewer the nematodes found in the soil. It was probable that most of the stem nematodes migrated from the host plant tissue (Wallace, 1962; Webster, 1964). The temperature 2-3 weeks before the fall population peak was about 20 C while 2-3 weeks before the spring population peak it was about 10-15 C. Consequently, the temperature in the range of 10-20 C was suitable for the reproduction and the activity of this nematode. This temperature range was well in agreement with the results reported by Barker (1959), Seinhorst (1950), Wallace (1961), and Blake (1962). The negative correlation of the stem nematode count with moisture, and temperature deviation from 15 C may suggest that the nematode density in the summer months would be very low.

It is worthy to point out that the alfalfa plants were about 8 to 12 inches high when the stem nematode density reached its peak. The growing stage of the host plant may contribute to the influence of the stem nematode density in the soil. However, during the present investigation the alfalfa was cut on October 1, but the stem nematode density at the depth of 0-10 cm during this period was as low as 5-7 nematodes per 400 cc of soil and was further declining. Both the soil and air temperatures were lower than 10 C which was not suitable for this nematode and the soil moisture was well below 50 percent of field capacity during this period. It was probable that this low temperature and soil moisture caused the low nematode density during this period. Thus, temperature and moisture were likely to play a more important role

than the growing stage of the host plant in the influence on the stem nematode reproduction and its population density in soil.

In the winter period the population density was less than 2 stem nematodes per 400 cc of soil for all depths. This density may not be enough to allow a rapid increase of nematodes in the spring (Palo, 1962). Thus, the stem nematode likely overwintered in the alfalfa stubble in the egg stage instead of the soil where high moisture and low temperature caused high mortality of the nematodes.

SUMMARY AND CONCLUSIONS

1. The alfalfa stem nematode (Ditylenchus dipsaci (Kuhn) Filipjev.) has been increasing as a serious pest on alfalfa in Utah and other Western States. This experiment was initiated to measure the change in stem nematode population in the soil at different soil depths during the period of August 6, 1965, to June 25, 1966. A field located near Smithfield, Utah, containing a six year old stand of Ranger alfalfa grown on Millville silt loam soil and infested with the alfalfa stem nematode was selected for this study.

2. Soil samples (400 cc) were taken from the depths of 0-10, 10-20, 20-30, and 30-40 cm at two-week intervals. Nematodes in the soil samples were recovered by the sifting and gravity method and the Baermann funnel method (Thorne, 1961). Temperature and soil moisture contents at each sampling depth were also determined every sampling date.

3. Most of the nematodes in the soil were recovered from the upper 10 cm layer of soil. There were two population cycles. The first peak came between late August and early September while the second peak was obtained the middle of May. The fall population peak (50 nematodes/400 cc of soil) was substantially higher than the spring peak (20 nematodes/400 cc of soil). In the winter period the nematode density in the soil was consistently low. Less than 5 nematodes per 400 cc of soil were found. The nematode density decreased drastically with the increase of depth. Less than 6 and 3 nematodes per 400 cc of soil were found in the depths of 20-30 cm and 30-40 cm, respectively.

4. Multiple correlation analysis between the nematode count and (a) Soil moisture, (b) Season, (c) Deviation of soil temperature from 15 C, (d) Square of temperature deviation, (e) Time lag variable of 21 days, (f) Season x temperature deviation and (g) Season x (Temperature deviation)² were carried out with stepwise deletion of variables. Temperature and moisture were the most critical factors in influencing the number of nematodes in the soil. The influence of moisture decreased with the increase of soil depth. The highest density of this nematode in the soil was obtained when the soil temperature was about 15 C both in autumn and in spring. The higher the soil moisture content, the more nematodes were found in the soil.

5. There was possibly a downward migration of this nematode in the soil that resulted in the development of a population peak in the 10-20 cm depth 2-3 weeks later than in the 0-10 cm layer, both in autumn and in spring.

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